Amendments to the Specification

In order to provide an application that conforms to present patent office requirements as well as to provide clear incorporation of prior amendments, entry of the present substitute specification is respectfully requested. The substitute specification includes the present amendments following as well as prior amendments. No new matter is incorporated in the substitute specification.

Please delete pages i and ii of the original application, said pages representing a table of contents and recitation of priority. The original recitation of priority has been previously amended. Please insert the recitation of priority on page 1 of the application, immediately following the title as follows:

[0001] This is a divisional application of United States Patent Application Serial No. 08/659,235, filed June 5, 1996, now U.S. Patent No. 5,877,281, issued on March 2, 1999, which is a continuation-in-part of United States Patent Application Serial No. 08/480,229, filed June 7, 1995, now U.S. Patent No. 5,874,562, issued on February 23, 1999, each of which is incorporated herein in its entirety.

Please replace the paragraph running from line 8 – 24 of page -9- with the following amended paragraph:

[0022] Figure 2. Homology analysis between the deduced amino acid sequence of the putative *del-1* gene (m-del1) (SEQ ID NO: 1) and other proteins with "discoidin-like domains." Identical residues are boxed, conserved residues are ~~shaded~~ in bold (Geneworks, Intelligenetics, Mountain View, CA). m-*del-1* sequence (SEQ ID NO: 1) was derived from a trapped exon and mouse embryo cDNAs. Abbreviations: h-MFG, human milk fat globule protein (SEQ ID NO: 2); h-FV, human coagulation factor V (SEQ ID NO: 3); m-FVIII, mouse coagulation factor VIII (SEQ ID NO: 4); X-A5b1 (SEQ ID NO: 5) and X-A5b2 (SEQ ID NO: 6), b1 and b2 domains of Xenopus neuronal antigen A5; dis-I, discoidin I (SEQ ID NO: 7); consensus sequence (SEQ ID NO: 8).

Please replace the paragraph running from line 19 – 24 of page -15- with the following amended paragraph:

[0036] Figure 16. Immunostaining of Del-1 in the developing bone (vertebral column) of a 13.5 day mouse embryo. The ~~laquanae~~ lacunae within the bone are structures composed of extracellular matrix proteins and they are stained for Del-1.

Please replace the paragraph beginning at line 35 of page -28- with the following amended paragraph:

[0067] An alternative expression system which could be used to express *del-1* is an insect system. In one such system, *Autographa californica* nuclear ~~polyhidrosis~~ polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera*

frugiperda cells. The *del-1* coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the *del-1* coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (*e.g., see* Smith et al., 1983, *J. Viol.* 46:584; Smith, U.S. Patent No. 4,215,051). A commercially available baculovirus expression vector pFastBac 1 (Gibco BRL, Inc.) has been constructed to contain the murine *del-1* coding sequence.

Please replace the paragraph beginning at line 6 of page -44- with the following amended paragraph:

[00109] The anti-angiogenic activity of Del-1 may be used to treat abnormal conditions that result from angiogenesis. These conditions include, but are not limited to, cancer, diabetic retinopathy, rheumatoid arthritis and endometriosis. Additionally, the removal or inhibition of Del-1 in situations where it naturally inhibits blood vessel formation may be used to promote angiogenesis. These conditions ~~include~~ include, but are not limited to, cardiac ischemia, thrombotic stroke, wound healing and peripheral vascular disease. Furthermore, Del-1 may be used to stimulate bone formation.

Please replace the paragraph beginning at line 18 of page -63- with the following amended paragraph:

[00139] Several stably transfected yolk sac cell clones with the *del-1* gene were selected (Figure 14B). When the transfected cells were reacted with the aforementioned antibody, both the membrane of certain yolk sac cells and the extracellular matrix were stained as compared with mock-transfected yolk sac cells as negative control (Figure 15A, 15B). In keeping with this staining pattern, immunostaining of developing bone of a 13.5 day mouse embryo detected the Del-1 protein in the ~~laquanae~~ lacunae within the bone, which were composed of extracellular matrix proteins (Figure 16).

Please replace the paragraph beginning at line 16 of page -69- with the following amended paragraph:

[00158] A second experiment was conducted in which a probe that had previously been mapped to 5q34, and confirmed by cohybridization with a probe from the cri du chat locus which is known to localize to 5p15, was cohybridized with clone 10043. This experiment resulted in the specific labeling of the mid and distal long arm of chromosome 5 (Figure 21A and B). Measurements of 10 specifically hybridized chromosomes 5 demonstrated that clone 10043 was located at a position which was 29% of the distance ~~form~~ from the centromere to the telomere of chromosome arm 5q, an area that corresponded to band 5q14. A total of 80 metaphase cells were analyzed with 74 exhibiting specific labeling. This region of the chromosome has been found to be a break point in some human cancers (Wieland and Bohm,

1994, *Cancer Res.* 54:1772; Fong et al., 1995, *Cancer Res.* 55:220; Wieland et al., 1996, 12:97, *Oncogene* 12:97). Thus, chromosome 5 aberrations may lead to altered expression of *del-1* and contribute to the malignant phenotype.

Please replace the Abstract of page -99- with the following amended Abstract:

The present invention relates to a member of a novel gene family referred to as developmentally-regulated endothelial cell locus-1 (*del-1*). In particularly, the invention related to del-1 nucleotide sequences, Del-1 amino acid sequences, methods of expressing a functional gene product, and methods of using the gene and gene product. Since del-1 is expressed in endothelial cells and certain cancer cells, it may be useful as an endothelial cell and tumor marker. ~~In addition, the ability of Del-1 to inhibit vascular formation allows its use as an anti-angiogenic agent.~~ In addition, anti-Del-1 antibodies for immunological assessment of Del-1 protein expression as well as for inhibiting or stimulating Del-1 function are provided. [P0078, line 1 – 3 and P0079, line 4]

Amendments to the Sequence listing

A new sequence listing, including paper and disk copies and statement is support, is submitted herewith to correct errors in the prior submissions. The presently submitted sequence listing does not introduce new matter. Support for the amendments can be found in the specification and drawings as originally filled and as indicated below:

SEQ ID NO: 5. Discoidin-Like domain X-A5b1. The original and subsequently filed sequence listings erroneously recited the sequence shown on Figure 2. The sequence and point of correction are depicted below:

DLENLRFVSGIGTQGAISKETKKKYFVKSYKVDISSNGEDWITLK[[GD]]DGNKHLVFTG
NTDATDVVYRPFSKPVITRFVRLRPVTW

SEQ ID NO: 6. Discoidin-Like domain X-A5b2. The original sequence listing was correct in accordance with Figure 2, however an error was introduced in subsequent submissions. The sequence and point of correction are depicted below:

DLAEEKIVRGVIIQGGKHKENKVFMRKFKIGYSNNGTEW[[G]]EMIMDSSKNKPKTFEGN
TNYDTPELRTFAHITTGFIRIIP

SEQ ID NO: 7. Discoidin-Like domain hDis-1. The original sequence listing was correct in accordance with Figure 2, however an error was introduced in subsequent submissions. The sequence and point of correction are depicted below:

GCEVPRTFMCVALQGRGDADQWVTSYKIRYSLDNVSWFEYR[[D]]NGAAITGVTDRNTVV
NHFFDTPIRARSIAIHPLT

SEQ ID NO: 9. murine del-1 from Figures 3A-D. The sequence has not been changed but has been formatted via PatentIn3.2 to provide a sequence conversion to encoded amino acids.

SEQ ID NO: 11. Human Del-1 from Figure 11. The original and subsequently filed sequence listings erroneously recited the sequence shown on Figure 11. The sequence and point of correction are depicted below:

```
aga tgg [[aag]] aac cgg tgg att cag ata aat ttg caa aga aaa atg aga gtt      864
Arg Trp [[Lys]] Asn Arg Trp Ile Gln Ile Asn Leu Gln Arg Lys Met Arg Val
                270                 275                 280
```

SEQ ID NO: 14. Human Del-1 amino acid sequence from Figure 11. The original and subsequently filed sequence listings erroneously recited the sequence shown on Figure 11. The sequence and point of correction are depicted below:

```
Glu Asn Asp Arg Trp [[Lys]] Asn Arg Trp Ile Gln Ile Asn Leu Gln Arg Lys
                245                 250                 255
```

SEQ ID NO: 20. MFG-E8 from Figure 8. The original sequence listing was correct in accordance with Figure 8, however an error was introduced in subsequent submissions. The sequence and point of correction are depicted below:

```
Trp Tyr Pro His Leu Gly Arg Leu Asp Asn Gln Gly [[Leu]] Lys Ile Asn Ala
        195                 200                         205
```

SEQ ID NO: 21. Human Del-1 discoidin domains from Figure 8. The original sequence listing was correct in accordance with Figure 8, however an error was introduced in subsequent submissions. The sequence and point of correction are depicted below:

```
Ile Thr Ala Ser Ser Thr His Arg Ala Leu Phe Gly Leu Gln [[Leu]] Lys Trp
            20                  25                  30
```

SEQ ID NO: 24. Human Del-1 EGF Domain 2 from Figure 10. The original and subsequently filed sequence listings did not appear to include the final two amino acids of the Figure. The sequence and point of correction are depicted below:

```
Gly Phe Asn Gly Ile His Cys Gln His Asn Ile Asn Glu
        35                  40                  45
```

SEQ ID NO: 25. Human Del-1 EGF Domain 3 from Figure 10. The original sequence listing was correct in accordance with Figure 10, however an error was introduced in subsequent submissions. The sequence and point of correction are depicted below:

```
Ala Asn Tyr Ser Cys Glu Cys Pro Gly Glu Phe Met Gly Arg Asn Cys [[Glu]] Gln Tyr Lys

            20                  25                  30                      35
```

SEQ ID NO: 28. Murine truncated minor variant from Figure 12. The original sequence listing was correct in accordance with Figure 12, however an error was introduced in subsequent submissions. The sequence and point of correction are depicted below:

```
ggaacccaag gagccgccgt ccgcccgctg tgcctctgct agaccactcg cagccccagc    300

                      tccgcgcacc ccagctcagg cgaagctgga gtgagggtga
ctctctcaag cgcacccacc ~~accactttt tatcgccctt cccaagattt gagaagcgct~~    360

atcacccttt ctctagggcc accactcttt tatcgccctt cccaagattt gagaagcgct    420
```

SEQ ID NO: 30. Human Del-1 from Figure 6. The following errors were found in SEQ ID No: 30. The sequence and point of correction are depicted below:

```
Glu Asn Asp Arg Trp [[Lys]] Asn Arg Trp Ile Gln Ile Asn Leu Gln Arg Lys
    210                     215                 220

Thr Ser Gly His Asn Asp Gln Ser Gln Trp Leu Gln Val [[Asp]] Xaa Leu Leu
    370                 375                 380

Val Pro Thr Lys Val Thr Gly Ile Ile Thr Gln Gly Ala Lys Asp [[Phe]] Xaa
385                 390                 395                             400

Gly Glu His Trp Thr Val [[Tyr]] Xaa Gln Asp Glu Lys Gln Arg Lys Asp Lys
            420                     425                 430

Val [[Phe]] Xaa Gln Gly Asn Phe Asp Asn Asp Thr His Arg Lys Asn Val Ile
                435                 440                 445
```